frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 19 June 2025
DOI 10.3389/fpls.2025.1599610

OPEN ACCESS

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RECEIVED 25 March 2025
ACCEPTED 26 May 2025
PUBLISHED 19 June 2025

CITATION
Shi C, Li P, Li L, Qi Z, Lu T, Shi J, Yang H, Wu J, Guo J, Liu M and Wu X (2025) Characterization and functional analysis of the *BAM* gene family in peanut (*Arachis hypogaea* L.).
Front. Plant Sci. 16:1599610.
doi: 10.3389/fpls.2025.1599610

Characterization and functional analysis of the *BAM* gene family in peanut (*Arachis hypogaea* L.)

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β-Amylase (BAM) is a kind of amylase in plants and microorganisms, which plays an important role in regulating plant growth and development and stress response. This study conducted a genome-wide identification and analysis of the BAM gene family in peanuts, identifying a total of 18 *AhBAM* genes. The encoded proteins exhibited significant variations in length, molecular weight, and isoelectric points, with primary localization in chloroplasts and nuclei. These genes were unevenly distributed across 10 chromosomes, with chr05 and chr15 each containing three genes. Phylogenetic analysis classified them into four subfamilies, with motif 3 serving as a conserved domain, and segmental duplication identified as the primary mechanism for family expansion. Synteny analysis indicated a closer evolutionary relationship between cultivated peanuts and soybeans. Cis-acting element analysis revealed that *AhBAM* genes may participate in light signaling, hormone regulation, and stress responses. *AhBAM3* emerged as a key node within the protein-protein interaction network, then the GO analysis pinpointed starch metabolism and drought response as the primary functional enrichments for this gene family. Expression profiling showed that *AhBAM8* was highly expressed in multiple tissues, whereas most members exhibited no significant response to web blotch disease. This comprehensive analysis provides a holistic view of the potential functions of the *AhBAM* families in peanuts and lays the foundation for future experimental validation of their roles in enhancing peanut stress resistance and productivity.

KEYWORDS

peanut (*Arachis hypogaea* L.), β-amylase, gene family, bioinformatics analysis, expression pattern

Introduction

As an important allotetraploid oil and food crop, peanut (*Arachis hypogaea* L.) is rich in plant nutrients such as isoflavones, phenolic acids, and phytosterols, and is primarily used in oil and food processing (Toomer, 2018; Zhang et al., 2024). Peanuts not only contain a large amount of unsaturated fats but also are a source of plant proteins and dietary fiber, playing a significant role in human health (Toomer, 2018). In recent years, with the successive deciphering of the genomes of peanut diploid wild species and allotetraploid cultivated species (Lu et al., 2024; Wang et al., 2025), the functional genomics of peanuts has made remarkable progress. In particular, the construction of the peanut three-dimensional genome chromatin organization map has been achieved. combined with joint analyses of Hi-C, ATAC-seq, and multi-tissue expression profiles, it has revealed the important regulatory role of chromatin spatial structure in peanut plant architecture (Zhang et al., 2021). These findings have provided new perspectives and theoretical foundations for a deeper understanding of the genetic characteristics of peanuts and their applications in agricultural production. Gene families, as collections of functionally related genes in the genome, are the core basis for plant adaptation to the environment, evolution, and developmental regulation. A deep analysis of the composition, structure, and function of peanut gene families will not only help reveal their unique biological characteristics but also provide theoretical support for the genetic improvement and sustainable development of peanuts.

Starch is not only the primary storage carbohydrate in plants, but also plays a crucial role in plant responses to abiotic stresses, such as drought, salt stress, and extreme high temperatures (Chen et al., 2022). β-Amylase (BAM) is a type of amylase widely found in plants and microorganisms, capable of catalyzing the hydrolysis of starch to produce maltose and other oligosaccharides. In plants, the activity of BAM is significantly influenced by external environmental factors such as drought, cold, and high temperatures (Monroe and Storm, 2018). In recent years, the *BAM* genes have been identified to possess diverse biological functions in various plant species, including *Arabidopsis*, maize, and cotton, and the signaling pathways and transcriptional regulatory networks in which they are involved are gradually being elucidated (David et al., 2022; Ravenburg et al., 2022; Yang et al., 2023). Although the *BAM* genes exhibit a certain degree of conservation across different plant species, significant differences in expression patterns and functions still exist among species due to interspecific variations.

In upland cotton (*Gossypium hirsutum*), 27 *GhBAM* genes have been identified, with *GhBAM7* implicated in regulating fiber strength during development (Yang et al., 2023). In addition, in maize (*Zea mays*), *ZmBAM8* demonstrates significant induction under drought, osmotic stress, and abscisic acid treatment, exhibiting chloroplast-localized activity that aligns with starch metabolism dynamics. The recombinant *ZmBAM8* protein shows high starch-hydrolyzing efficiency, and its overexpression enhances starch degradation and drought tolerance (Niu et al., 2024). Except drought responses, *BAMs* participate in cold stress adaptation in rice (*Oryza sativa*), OsMYB30 transcriptionally represses *BAM* genes, modulating starch-derived maltose accumulation to influence cold tolerance (Lv et al., 2017). Similarly, in pomegranate (*Punica granatum*), eight *PgBAM* genes contribute to pericarp development and cold stress response, with *PgBAM4* regulated by the cold-induced transcription factor *PgCBF7* (Liu et al., 2024). Jujube (*Ziziphus jujuba*) possessed nine *ZjBAM* genes, four of which (*ZjBAM1/2/5/6*) are drought-responsive and interact with α-amylase and glucanotransferase (Ma et al., 2022). These findings highlight the conserved yet specialized roles of *BAMs* in stress adaptation and developmental processes, and these studies not only provide important insights into the functional mechanisms of the *BAM* gene family but also offer potential targets for improving stress resistance and quality traits in crops through genetic engineering, holding significant theoretical and practical value.

In summary, the *BAM* gene family plays an important role in plant growth, development, and stress responses. However, the specific functions of *BAM* genes in peanut have not yet been studied. Based on this, the current study identified and characterized the peanut *BAM* gene family at the whole-genome level. Using bioinformatics approaches, we analyzed the gene structure, conserved motifs, cis-acting elements, and three-dimensional structures of peanut *BAM* family members. Moreover, by integrating transcriptome data, we further explored the potential biological functions of *BAM* family members. This study provides a theoretical basis for understanding the molecular mechanisms underlying peanut growth, development, and stress resistance and offers important targets for the development of new peanut varieties.

Materials and methods

Plant material and treatments

The phytohormone treatments were performed using distinct peanut cultivars - Baihua 15 for methyl jasmonate (MeJA) and Yuanza 9102 for salicylic acid (SA) - in the controlled environment of Zhengzhou University of Light Industry's plant growth greenhouse under an 8h light/16h dark photoperiod. For each treatment, 0.5 mM hormone solutions were carefully applied to both leaf surfaces of uniformly-grown plants using standardized spraying techniques. Tissue sampling followed precise temporal patterns: MeJA-treated leaves were collected at 0h, 4h, and 12h post-application, while SA-treated samples were taken at 0h, 4h, 12h, and 24h intervals. All harvested leaf tissues underwent immediate liquid nitrogen flash-freezing followed by thorough grinding for total RNA extraction. After quality verification, high-quality RNA served as template for cDNA synthesis using the PrimeScript RT reagent kit, with the resulting cDNA subsequently employed for quantitative real-time PCR (qRT-PCR) validation analyses conducted in triplicate biological replicates. A total of seven candidate genes were validated for their expression patterns

under MeJA and SA treatments using qRT-PCR analysis. Primers were shown in Supplementary Table S1.

Identification and chromosomal localization of peanut *BAM* family members

The genome data of peanut were obtained from the peanut genome database (https://www.peanutbase.org/). A hidden Markov model of the typical BAM family protein structure was downloaded from the Pfam database (http://pfam.xfam.org) (Mistry et al., 2021), and search for the BAM domain (PF01373) through the HMMER 3.1 software to initially obtain the members of the peanut *BAM* family (Krejci et al., 2016). The candidate proteins identified by the aforementioned methods were submitted to the SMART (http://smart.embl-heidelberg.de) and NCBI CDD (https://www.ncbi.nlm.nih.gov/cdd/) databases for manual identification and screening to ascertain the ultimate members of the peanut *BAM* family (Lu et al., 2020; Letunic et al., 2021). Finally, 18 *AhBAM* genes were obtained. The BioPerl tool was used to predict protein physicochemical parameters (Stajich, 2007). Subcellular localization predictions were generated with WoLF PSORT (https://wolfpsort.hgc.jp/) (Horton et al., 2007). Subsequently, the locations of 18 *AhBAM* genes on chromosomes were obtained based on the information annotated for the peanut genome and analyzed through the Gene Location Visualization of TBtools (Chen et al., 2023a).

Phylogenetic analysis, gene structure analysis and conserved motif analysis

The phylogenetic trees of peanut and its two ancestral species (*Arachis duranensis, Arachis ipaensis*), along with those of peanut, *Arabidopsis* and soybean, were respectively constructed by using the maximum likelihood method via IQTREE (Lam-Tung et al., 2015). The obtained evolutionary tree was submitted to the online website ITOL (https://itol.embl.de/) for beautification (Letunic and Bork, 2021). And the gene structure information of *BAM* family members was extracted from the peanut genome annotation file. The MEME online website (http://meme-suite.org) was used to predict the conserved domains in *AhBAM* protein sequences (Bailey et al., 2015). For this analysis, the number of motifs to be identified was set to 10, while default settings were adopted for other parameters and the results were visualized using TBtools.

Gene duplication, and synteny analysis

Arachis duranensis, Arachis ipaensis, *Arabidopsis* and soybean downloaded from NCBI. To investigate the evolutionary patterns of *AhBAM* genes, we conducted comprehensive synteny analysis using MCscanX with the following analytical pipeline: First, the software identified microsynteny blocks through rigorous BLASTP alignments, enabling precise detection of both segmental and tandem duplication events within the *AhBAM* gene family. Subsequently, the algorithm established macrosyntenic relationships between peanut and its evolutionary relatives (*Arabidopsis thaliana* and *Glycine max*) by comparing conserved gene collinearity across genomes. For intuitive visualization of these complex genomic relationships, we employed Circos to generate integrated circular plots where: (i) distinct color schemes differentiated individual chromosomes, (ii) gracefully curved connectors highlighted orthologous gene pairs among species, and (iii) multi-layer heatmaps simultaneously displayed quantitative data including gene expression profiles and duplication classifications (Krzywinski et al., 2009; Wang et al., 2012).

Subsequently, to evaluate the evolutionary selection pressures acting on the duplicated *AhBAM* gene pairs, we employed KaKs Calculator 2.0 (Wang et al., 2010) for detailed sequence divergence analysis. This sophisticated computational tool implements multiple nucleotide substitution models (including NG, LWL, and YN methods) to accurately calculate: (1) synonymous substitution rates (Ks); and (2) nonsynonymous substitution rates (Ka). This analysis provided critical insights into the evolutionary forces shaping the functional diversification of *AhBAM* genes following duplication events.

Cis–acting element analysis of *AhBAM* genes

The 2000-bp upstream promoter regions of the *AhBAM* genes were extracted using TBtools software and analyzed for cis-regulatory elements through the PlantCARE online platform (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot et al., 2002). First, TBtools was employed to precisely isolate regulatory regions from -2000 to -1 bp relative to the transcription start site (TSS) of each *AhBAM* gene. The obtained FASTA-formatted sequences were then submitted to the PlantCARE database, then utilizing Position Weight Matrix (PWM) algorithms to identify key cis-regulatory elements such as light-responsive elements (e.g., G-box), and hormone-responsive elements (e.g., ABRE). Finally, manual verification was performed to screen prediction reliability, then the TBtools software was used to visualize the cis-regulatory elements.

Protein–protein interaction network construction

The obtained *AhBAM* protein sequences were constructed using string database (https://string-db.org/) with the following parameters: organism set to *Arachis hypogaea*, medium confidence score (0.400), and full network type. The resulting protein–protein interaction (PPI) network was downloaded as a TSV file and imported into Cytoscape (v3.10.3) for advanced visualization, where node size represented interaction degree and

edge thickness indicated confidence scores (Shannon et al., 2003; Szklarczyk et al., 2023).

Gene Ontology analysis of *AhBAM* genes

The complete peanut protein sequence were analyzed using EGGNOG-MAPPER (http://eggnog-mapper.embl.de/) with default parameters to generate to generate comprehensive functional annotation files, including COG/KOG classifications and potential orthologous groups (Huerta-Cepas et al., 2019). For GO enrichment analysis of *AhBAM* genes, we employed TBtools with Fisher's exact test (FDR<0.05) to identify significantly enriched terms across three ontologies: biological process, molecular function, and cellular component. The enrichment results were then visualized through the Weishengxin online platform (https://www.bioinformatics.com.cn/) for visualization (Tang et al., 2023).

Prediction of phosphorylation, acetylation and methylation sites

The *AhBAM* protein sequence was submitted to the GPS 6.0 (https://gps.biocuckoo.cn/) for prediction of phosphorylation, acetylation and methylation modification sites (Chen et al., 2023b). To reduce the impact of false positives on the prediction results, the threshold was set to high. However, under the high threshold condition, the prediction results of methylation sites were too few. Therefore, the prediction threshold of methylation sites was set to medium. The prediction results were manually screened according to the score to obtain the final results. Visualize the prediction results using the IBS 2.0 (https://ibs.renlab.org/#/home) (Xie et al., 2022).

Three-dimensional and secondary structure prediction

We utilized the SWISS-MODEL (https://swissmodel.expasy.org/) website to construct the three-dimensional structure model of the target protein by aligning the target protein sequence with solved protein structure templates based on known protein structure information (Waterhouse et al., 2018). We further utilized the GOR4 (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html) to predict the secondary structure of the target protein, and Its principle is based on statistical analysis and pattern recognition, by analyzing the amino acid sequence patterns in known protein structures.

Expression patterns of *AhBAM* genes and qRT-PCR Validation

We conducted a comprehensive transcriptomic analysis of AhBAM genes using peanut genomic resources, examining both tissue-specific expression and stress responses. From the Peanut Genome Database, we analyzed RNA-seq data across eight tissues (root, stem, leaf, flower, peg, pod, and early/mature seeds) (Clevenger et al., 2016). Then analyzed RNA-seq data of peanut web blotch infection (Wu, 2024).

Results

Genome-wide identification and chromosomal distribution of the *BAM* gene family

A total of 18 *AhBAM* genes were identified and characterized from the peanut genome. According to their chromosomal positions, these genes were sequentially renamed from *AhBAM1* to *AhBAM18* (Table 1). The proteins encoded by these genes exhibit diverse lengths, with amino acid counts ranging from 229 residues in *AhBAM7* to 1144 residues in *AhBAM5*. Their relative molecular weights (MW) vary from 25,224.6 Da (*AhBAM7*) to 128,503.9 Da (*AhBAM5*), and their theoretical isoelectric points (pI) range from 5.48 (*AhBAM11*) to 9.17 (*AhBAM1*). Subcellular localization analysis revealed that the majority of AhBAM proteins are targeted to the chloroplast and nucleus, with seven members each. Two members are localized to the peroxisome, while the extracellular matrix and endoplasmic reticulum each contain one *AhBAM* gene. Based on these findings, we speculate that *AhBAM* family members may primarily performed their functions within the nucleus and chloroplast, where they could play key roles in regulating gene expression and metabolic processes.

The chromosomal localization analysis revealed that the *AhBAM* genes are unevenly distributed across 10 chromosomes in the peanut genome. Specifically, chromosomes 5 (chr05) and 15 (chr15) harbor the highest number of *AhBAM* genes, each containing three genes. Chromosomes 3 (chr03), 4 (chr04), 13 (chr13), and 14 (chr14) each possess two *AhBAM* genes, while chromosomes 1 (chr01), 8 (chr08), 11 (chr11), and 18 (chr18) each contain only a single *AhBAM* gene (Figure 1). This uneven distribution pattern suggests potential functional divergence and evolutionary dynamics of the *AhBAM* gene family within the peanut genome.

Phylogenetic, gene structural, and conserved motif analyses

The protein sequences of the 18 identified AhBAM genes were used to construct phylogenetic trees in conjunction with those from two ancestral species (AdBAM and AiBAM), as well as from Arabidopsis (AtBAM) and soybean (GmBAM) (Figures 2A, B). The analysis revealed that the AhBAM genes are classified into four distinct subfamilies. Notably, the largest subfamily comprises six members of the peanut BAM gene family, while the remaining three subfamilies each contain four members.

The conserved motif analysis within the *AhBAM* gene family revealed that Motif 3 is present in all members and represents a

TABLE 1 The information of *BAM* family members in peanut.

Gene Name	Gene ID	Chromosome localization	Amino acid length(aa)	MW(Da)	PI	Subcellular localization
AhBAM1	arahy.3VMA9Y	chr01	575	64652	9.17	Chloroplast
AhBAM2	arahy.NQ88PQ	chr03	559	62965.2	6.16	Chloroplast
AhBAM3	arahy.U96YPL	chr03	577	65146.7	8.72	Chloroplast
AhBAM4	arahy.I36EUT	chr04	533	58374.6	6.51	Nucleus
AhBAM5	arahy.UANN5Y	chr04	1144	128503.9	6.21	Nucleus
AhBAM6	arahy.K0N155	chr05	647	73093.8	6.44	Nucleus
AhBAM7	arahy.68PBWJ	chr05	229	25224.6	7.16	Nucleus
AhBAM8	arahy.4Y2508	chr05	585	64583.7	6.7	Peroxisome
AhBAM9	arahy.C3I2PC	chr08	586	66246	7.77	Chloroplast
AhBAM10	arahy.34GL3D	chr11	544	60998.7	8.72	Chloroplast
AhBAM11	arahy.0V89VS	chr13	468	52658.7	5.48	Extracellular matrix
AhBAM12	arahy.ABG1ZW	chr13	559	63106.3	8.45	Chloroplast
AhBAM13	arahy.60DV95	chr14	533	58330.5	6.36	Nucleus
AhBAM14	arahy.M409IX	chr14	1109	124435	5.99	Nucleus
AhBAM15	arahy.Y0TSMC	chr15	647	73093.8	6.44	Nucleus
AhBAM16	arahy.JJTS0M	chr15	482	53301.1	6.68	Endoplasmic reticulum
AhBAM17	arahy.TP5BL7	chr15	585	64664.7	6.45	Peroxisome
AhBAM18	arahy.Y6Z03A	chr18	606	68605.8	7.36	Chloroplast

typical conserved domain of the *AhBAM* family. Additionally, 17 members contain both Motif 2 and Motif 5, while 16 members conclude Motif 1, Motif 4, Motif 6, Motif 7, and Motif 9. Among all *AhBAM* members, *AhBAM5* and *AhBAM14* possess all identified motifs, whereas *AhBAM16* contains only two motifs (Figures 3A, B). Members of the same subfamily exhibit similar gene structures. For instance, G1 subfamily members uniformly possess a higher number of introns, with *AhBAM5* and *AhBAM14* having the most (Figure 3C). This pattern suggests that *AhBAM* genes within the same subfamily generally share similar conserved motifs and gene structures, likely reflecting functional and evolutionary conservation within these groups.

Gene duplication and synteny analysis

Among the peanut *BAM* family members, a total of eight pairs of large fragment duplicated genes were identified: *AhBAM11*: *AhBAM2*, *AhBAM10*: *AhBAM1*, *AhBAM8*: *AhBAM17*, *AhBAM13*: *AhBAM4*, *AhBAM12*: *AhBAM3*, *AhBAM9*: *AhBAM18*, *AhBAM6*: *AhBAM15*, and *AhBAM14*: *AhBAM5* (Figure 4A). This finding suggests that segmental duplication has been a primary mechanism driving the expansion of the peanut *BAM* gene family. Calculation of the nonsynonymous (Ka) and synonymous (Ks) substitution rates for each duplicated gene pair revealed that the Ka/Ks ratios of these *AhBAM* family members ranged from 0.063 to 0.392 (Table 2). These low Ka/Ks ratios indicate that these genes have undergone strong purifying selection, suggesting functional conservation and constraint during their evolutionary history.

The collinearity analysis among different species revealed that 16 pairs of homologous gene pairs were identified between cultivated peanuts and *Arachis duranensis*, while 15 pairs were identified between cultivated peanuts and *Arachis ipaensis* (Figure 4B). Additionally, comparisons between cultivated peanuts and other species showed that five homologous gene pairs were identified with *Arabidopsis thaliana*, and 34 pairs were identified with Glycine max (Figure 4C). These findings suggest that cultivated peanuts share a closer evolutionary relationship with soybeans, with many homologous gene pairs likely predating the divergence of their ancestral lineages. This highlights the potential conservation of key genetic elements across these species, which may have contributed to their shared adaptive traits and functional characteristics.

Analysis of cis-acting elements in *AhBAM* genes

The cis-acting elements within gene promoters directly reflect the potential functions of the corresponding genes. To elucidate the regulatory mechanisms of *AhBAM* genes, we analyzed the genomic sequences of the upstream regions (2000 base pairs) of

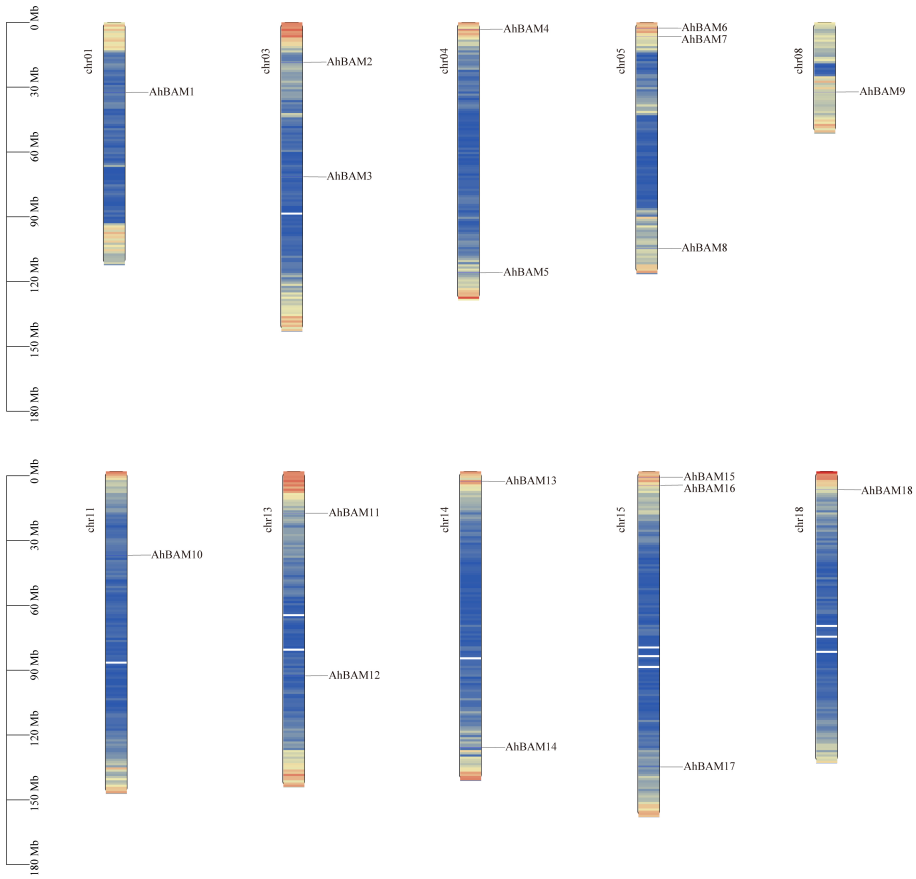

FIGURE 1
Chromosomal locations of *AhBAM* genes. The scale on the left presents the length of chromosomes (Mb).

these genes in the peanut genome. We identified and visualized 10 abundant cis-acting elements (Figure 5). Our analysis revealed that the promoters of *AhBAM* genes contain three main types of cis-acting elements: those involved in development, abiotic stress response, light response, and hormone response. Specifically, light-responsive elements include Box4, GATA-motif, G-Box, and GT1-motif; hormone-responsive elements include ABRE, CGTCA-motif, and TGACG-motif; and the LTR element is associated with abiotic stress response. These findings suggest that *AhBAM* genes may primarily function in light signaling, hormone regulation, and abiotic stress response pathways in peanut plants.

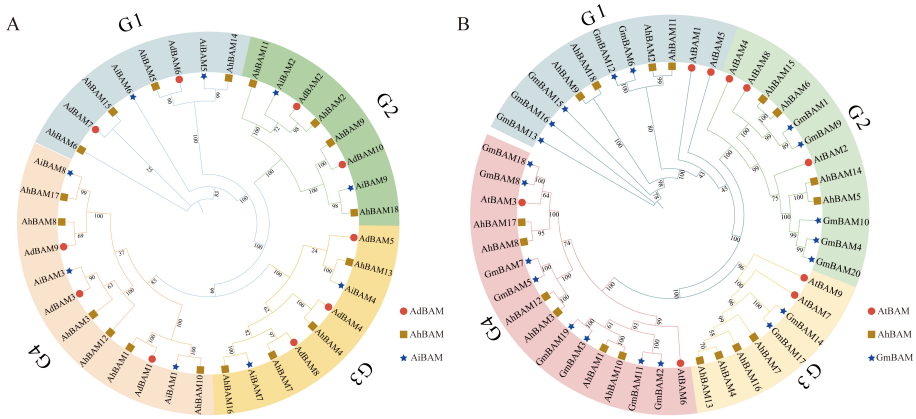

FIGURE 2
Phylogenetic analysis of the BAM family members. **(A)** Phylogenetic trees of BAM proteins for peanut, *Arachis duranensis* and *Arachis ipaensis*. **(B)** Phylogenetic trees of BAM proteins for peanut, *Arabidopsis* and soybean.

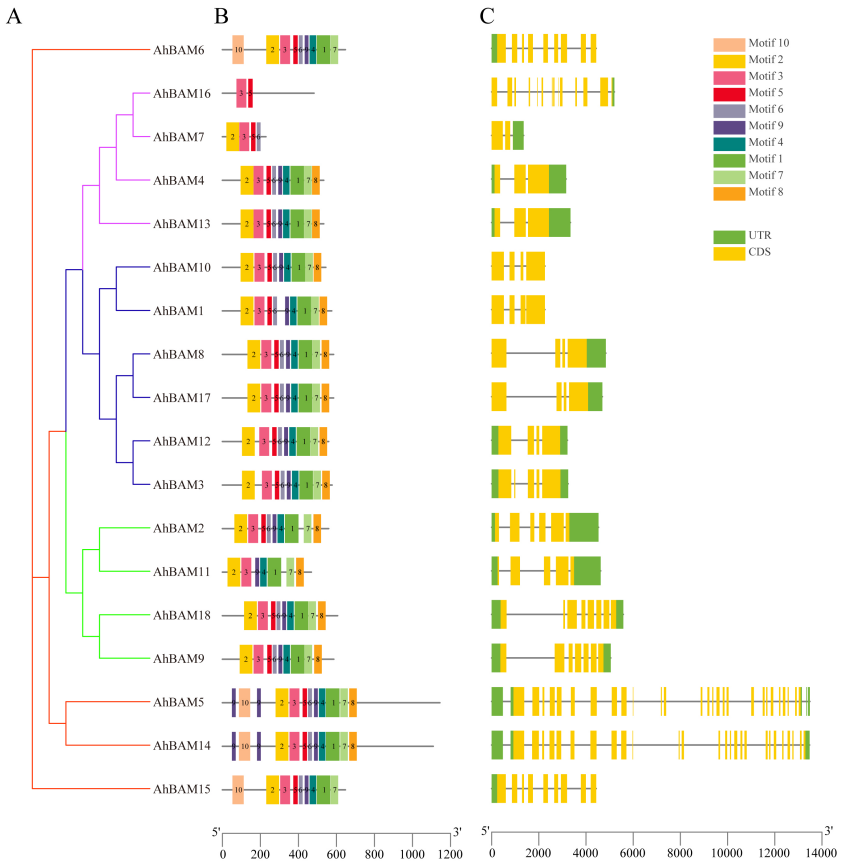

FIGURE 3
Phylogenetic tree, conserved motif, and gene structure of the *AhBAM* family in peanut. **(A)** Phylogenetic relationships of *AhBAM*. Different subgroups were marked with different colors. **(B)** Conserved motifs for *AhBAM* proteins in peanut. Different motifs are showed with different colored boxes and numbers (1–10). **(C)** The genetic structure of the *AhBAM* gene, including introns (black line), exons (yellow rectangle), and untranslated regions (UTRs, green rectangle). The scale bar of bottom demonstrates the length of exons and introns.

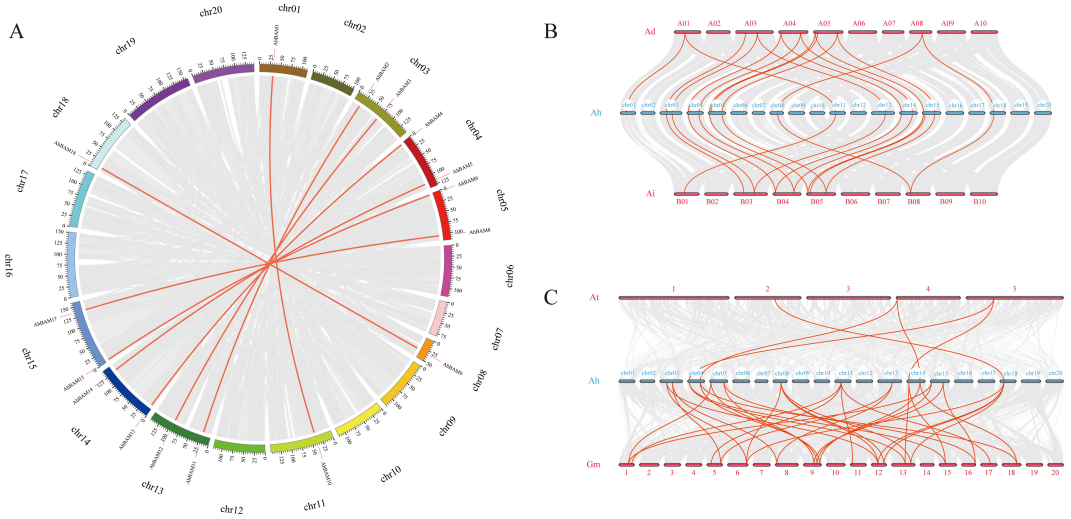

FIGURE 4
Gene duplication events in the *BAM* family. **(A)** Distribution and collinearity of the *AhBAM* gene family in the peanut genome. The gray background lines represent a collinear background and the red lines indicate a collinear relationship between *AhBAM* members. **(B)** Syntenic analysis of *BAM* genes among peanut, *Arachis duranensis* and *Arachis ipaensis*. **(C)** Syntenic analysis of *BAM* genes among peanut, *Arabidopsis* and soybean.

TABLE 2 Ka/Ks of orthologous gene pairs.

Orthologous gene pairs	Ka	Ks	Ka/Ks
AhBAM11/AhBAM2	0.007	0.041	0.173
AhBAM10/AhBAM1	0.002	0.037	0.063
AhBAM8/AhBAM17	0.008	0.039	0.219
AhBAM13/AhBAM4	0.009	0.026	0.336
AhBAM12/AhBAM3	0.014	0.035	0.392
AhBAM9/AhBAM18	0.009	0.035	0.274
AhBAM6/AhBAM15	NA	NA	NA
AhBAM14/AhBAM5	0.006	0.030	0.199

Protein-protein interaction network analysis and visualization

We constructed a protein-protein interaction (PPI) network, identifying 10 AhBAM proteins that formed 13 nodes connected by 39 distinct interaction edges (Figure 6). These interactions reveal a complex network of connections, highlighting the complex regulatory roles of these proteins. Notably, AhBAM3 emerged as the central hub within the network, connecting to nine other genes. The hub status of AhBAM3 strongly indicates its function as a central regulator, coordinating the activity of other AhBAM proteins and serving as a potential integration node for multiple signaling pathways and regulatory mechanisms.

GO analysis of *AhBAM* genes

Gene Ontology (GO) analysis of *AhBAM* genes revealed significant enrichment in specific functional categories (Figure 7). At the molecular function level, *AhBAM* genes are predominantly associated with amylase activity, consistent with their role in carbohydrate metabolism. In biological processes, these genes are highly enriched in starch catabolism and glucose catabolism, further supporting their involvement in energy mobilization and metabolic homeostasis. Additionally, *AhBAM* genes also showed significant enrichment in responses to water deprivation and cellular water homeostasis, suggesting that *AhBAM* genes may play a crucial role in mediating plant responses to drought adaptation and stress-related physiological regulation. These findings indicates that *AhBAM* genes may contribute to plant survival under water-limited conditions by modulating metabolic and stress-responsive pathways.

Prediction of post-translational modification sites: phosphorylation, acetylation, and methylation

Bioinformatic analysis predicted multiple post-translational modification sites (PTMs) in AhBAM family proteins, including phosphorylation, acetylation, and methylation. Then tyrosine phosphorylation sites were particularly abundant within these proteins (Figure 8A). Among them, AhBAM1 and AhBAM5 contained the highest number (13) of phosphorylation sites, while AhBAM16 showed the fewest (2). Acetylation site prediction identified nine AhBAM proteins as potential targets for this modification (Figure 8B). Acetylation, a critical post-translational modification, can significantly modulate protein structure and function, these modifications may play key regulatory roles in various cellular processes. Additionally, methylation sites analysis revealed eight AhBAM proteins as likely substrates for methylation (Figure 8C), indicating additional regulatory complexity.

Prediction of three-dimensional and secondary structures

Predicted three-dimensional (3D) structures of AhBAM proteins demonstrated sequence identity values ranging from 73.09% to 100% (Figure 9). AhBAM1, AhBAM3, and AhBAM9

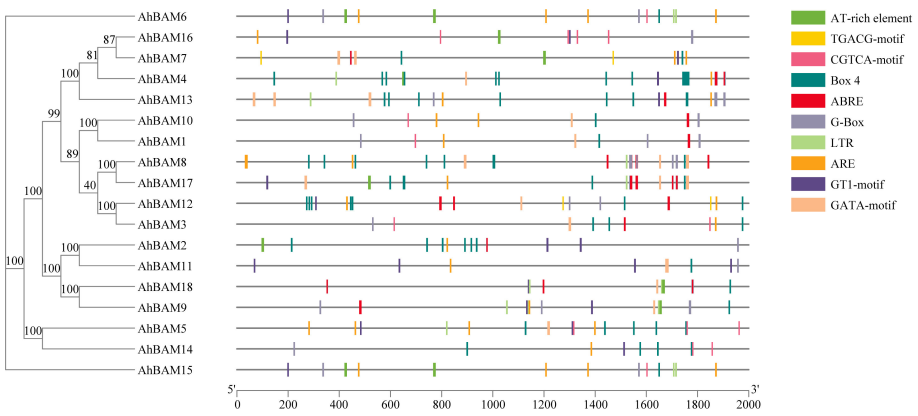

FIGURE 5
Distribution of cis-acting elements identified in the 2000 bp upstream promoter region of the peanut *AhBAM* gene.

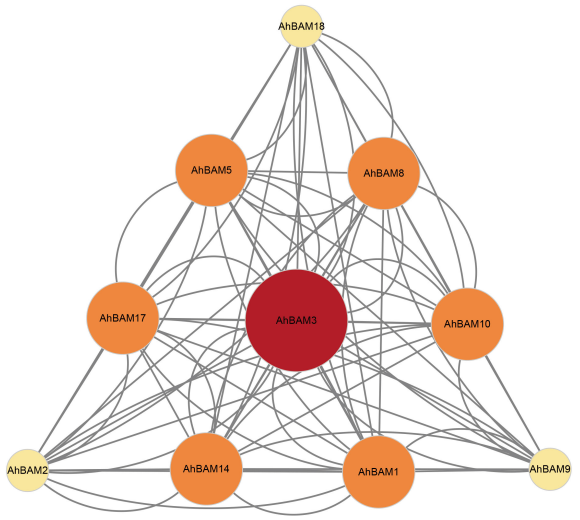

FIGURE 6
Protein–protein interaction (PPI) network of significant genes in peanut. Nodes represent proteins, central nodes are indicated in red, and black lines indicate interactions between nodes. The darker the color, the more important the protein in the interaction network.

exhibited the highest sequence identity, while AhBAM2 showed the lowest conservation. The structural reliability, as assessed by global model quality estimation (GMQE) scores, ranged from 0.40 to 0.91, indicating high-confidence predictions suitable for functional interpretation. Secondary structure analysis confirmed the critical structural role of α-helices (right-handed coiled structures stabilized by backbone hydrogen bonds) in determining protein conformation and function. The predicted α-helices composition ranged from 12.23% to 35.85%, suggesting these secondary structure elements may contribute to functional specialization through their impact on protein folding and domain architecture (Figure 10).

Expression pattern of *AhBAM* family members

Our research of *AhBAM* gene expression in seven peanut tissues (leaves, roots, flowers, branches, reproductive buds, pistils, and stamens) revealed distinct tissue-specific expression profiles (Figure 11A). Specifically, *AhBAM8* and *AhBAM18* exhibited higher expression in leaves, while *AhBAM4*, *AhBAM8*, *AhBAM13*, and *AhBAM17* were preferentially expressed in flowers. *AhBAM8* and *AhBAM17* exhibited particularly high transcript levels in pistils. In contrast, other tissues maintained relatively low expression of *AhBAM* genes (Figure 11A). The consistent high expression of *AhBAM8* in multiple tissues (leaves, flowers, and pistils) revealed its fundamental role in both vegetative and reproductive development of peanut.

Based on previous study, we performed the analysis of *AhBAM* gene expression in response to web blotch disease in peanuts. Results indicated that most *AhBAM* genes exhibited no significant transcriptional changes upon pathogen infection, with the

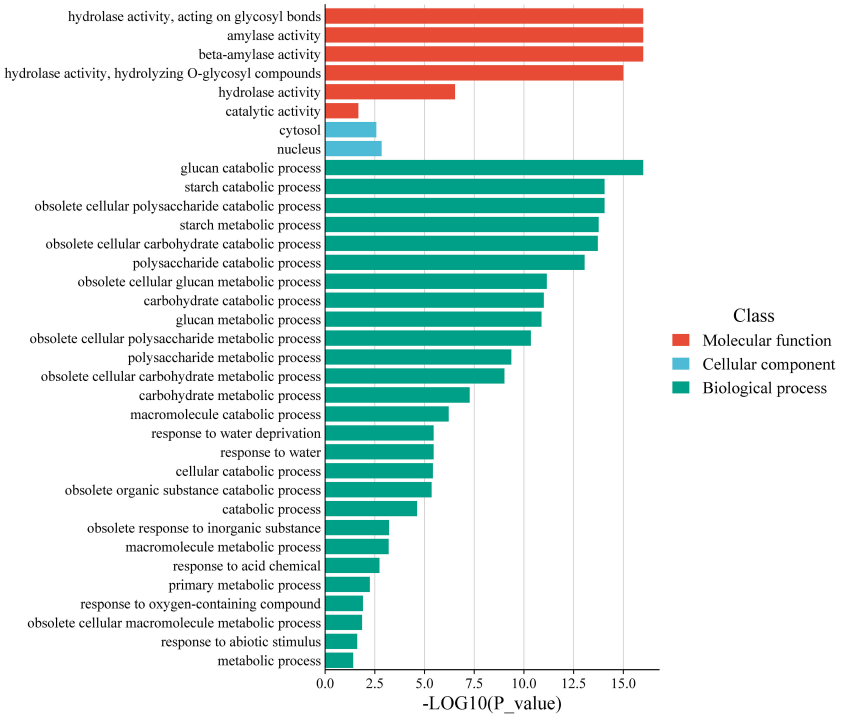

FIGURE 7
Histogram of *AhBAM* genes GO enrichment. The horizontal coordinate indicates the corrected p value, and the vertical coordinate indicates the GO term.

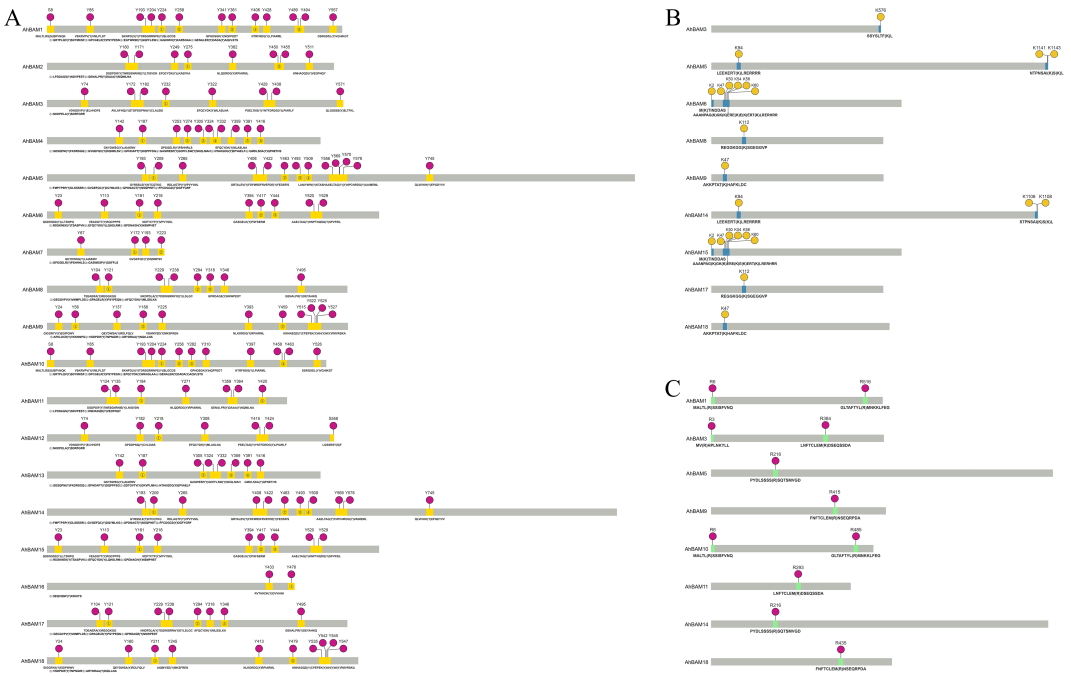

FIGURE 8
Prediction of phosphorylation, acetylation and methylation sites. **(A)** Phosphorylation site prediction of *AhBAM* gene family proteins. Red solid circle indicates the predicted phosphorylation sites, and yellow region indicates the peptide segments where the sites are located. **(B)** Acetylation site prediction. Yellow highlights the predicted acetylation sites, while blue marks the peptide segments containing these sites. **(C)** Methylation Site Prediction. Red highlights the predicted methylation sites, while green marks the corresponding peptide segments.

exception of AhBAM10, which showed moderately elevated expression (Figure 11B). These findings suggest that *AhBAM* genes are unlikely to play a major role in the defense response against web blotch disease, though the specific function of *AhBAM10* needs further investigation.

Our screening of publicly available expression datasets revealed seven AhBAM genes exhibiting putative stress-response characteristics. To investigate the roles of these genes in plant stress resistance mechanisms, we examined their dynamic expression patterns under treatments with core stress hormones (MeJA and SA). The results demonstrated that different AhBAM genes exhibited distinct temporal response patterns to hormonal stimuli: in the MeJA treatment group, AhBAM4, AhBAM13, and AhBAM17 functioned as early-response genes (showing >2-fold upregulation at 4 hours), while AhBAM1, AhBAM3, and AhBAM10 displayed late-response characteristics (>2-fold upregulation at 12 hours) (Figure 12A). However, SA treatment induced a different regulatory pattern: except for AhBAM3 which showed specific upregulation at 12 hours, four genes including AhBAM1 and AhBAM4 exhibited significant downregulation at the early stage (4 hours) (Figure 12B). These results demonstrate distinct functional specialization of AhBAM genes in phytohormone responses, with AhBAM3 emerging as a particularly noteworthy candidate due to its putative role in cross-talk between multiple stress response mechanisms.

Discussion

Genome-wide identification of the *BAM* gene family in different species

β-Amylases are evolutionarily conserved enzymes that catalyze the production of maltose and participate in plant metabolism and stress responses (Thalmann et al., 2019). The *BAM* gene family exhibits significant subfamily differentiation and functional diversity during evolution. Studies have shown that *BAM* genes in upland cotton, (27 *GhBAM* genes, 3 subfamilies) and jujube (9 *BAM* genes, 4 subfamilies), primarily regulate basic metabolic processes (Yang et al., 2023; Ma et al., 2022). Whereas those in pomegranate (8 *PgBAM* genes) and Chinese white pear (17 *PbBAM* genes) have evolved distinct stress-responsive functions, particularly in adaptation to cold and drought (Liu et al., 2024; Zhao et al., 2019). In this study, we identified 18 *AhBAM* genes in peanut, and classified into four subfamilies,. showing high consistency with species such as jujube and pomegranate. Futurermore, *AhBAM5* and *AhBAM14* from the G1 subfamily contain more than 24 introns, displaying complex structures similar to the stress-responsive *PbBAM* genes in pear. In contrast, *AhBAM4*, *AhBAM7*, and *AhBAM13* from the G3 subfamily possess only two introns, exhibiting a streamlined structure comparable to the *GhBAM* genes in cotton (Elsanosi et al., 2024). This cross-species structural-functional conservation suggests that

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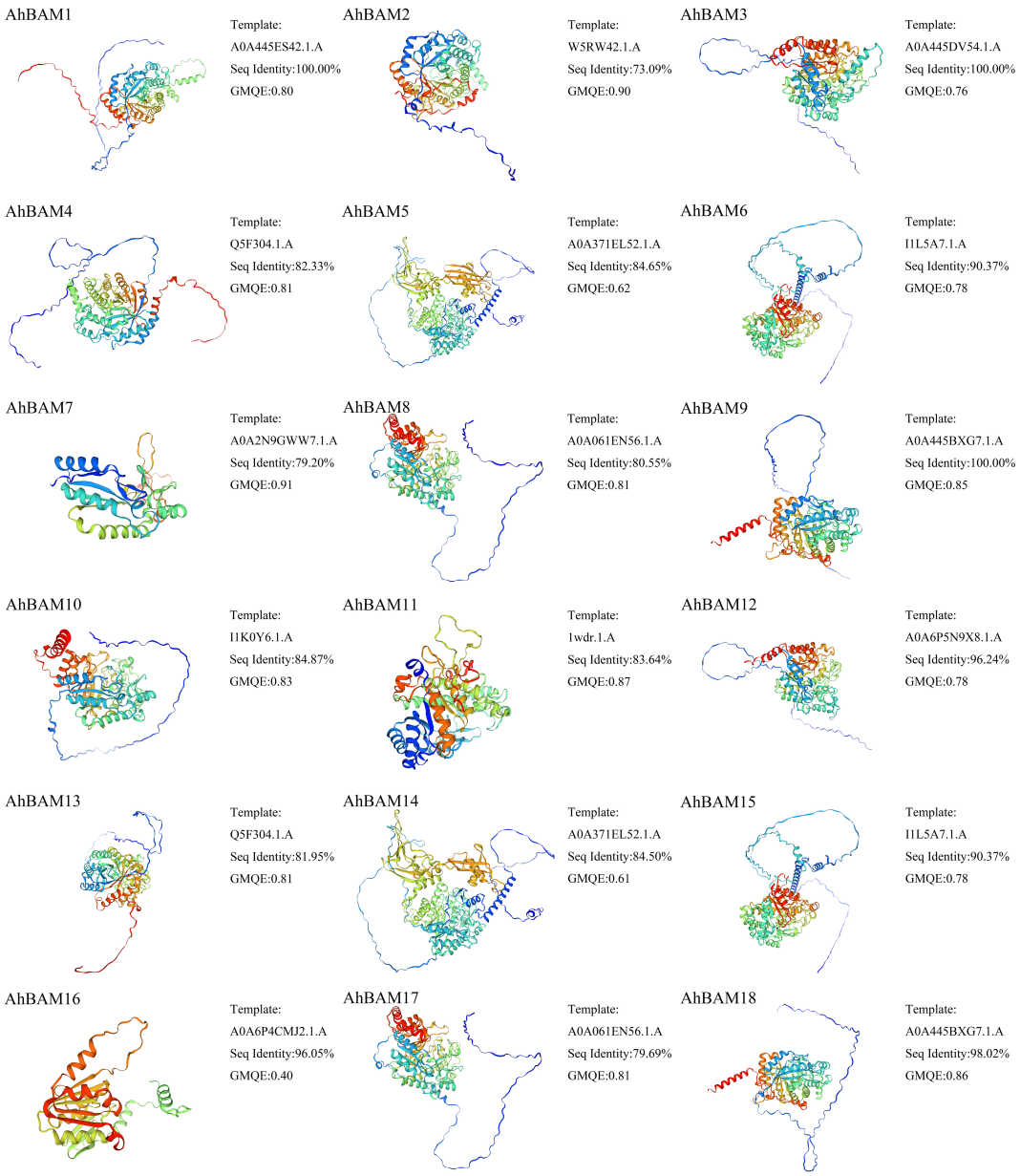

FIGURE 9
Three-dimensional structure prediction of AhBAM proteins.

peanut *BAM* genes may play dual roles in metabolic regulation and stress responses. Future research should focus on elucidating the molecular mechanisms of peanut *BAM* genes in abiotic stress responses, particularly the stress-adaptive functions of G1 subfamily genes, which could provide valuable candidate gene resources for breeding stress-resistant peanut varieties.

Gene duplication and selection pressure driving *AhBAM* family expansion

Gene duplication, including small-scale duplications (tandem and segmental duplications), serves as a crucial driving force in eukaryotic genome evolution (Kuzmin et al., 2022). In the peanut, *AhBAM* gene family, we identified eight segmentally duplicated gene pairs in the peanut *AhBAM* gene family, while no tandem duplications were detected. These findings in soybean and other leguminous plants may represent the primary mechanism for the expansion of the *BAM* gene family in legumes. Additionally, selection pressure analysis revealed that these duplicated gene pairs have undergone strong evolutionary selection, indicating their essential role in maintaining the conserved functions of *BAM* enzymes. Furthermore, synteny analysis between peanut and wild diploid relatives, as well as comparisons with other species, identified 34 syntenic gene pairs between peanut and soybean, a number significantly higher than that observed

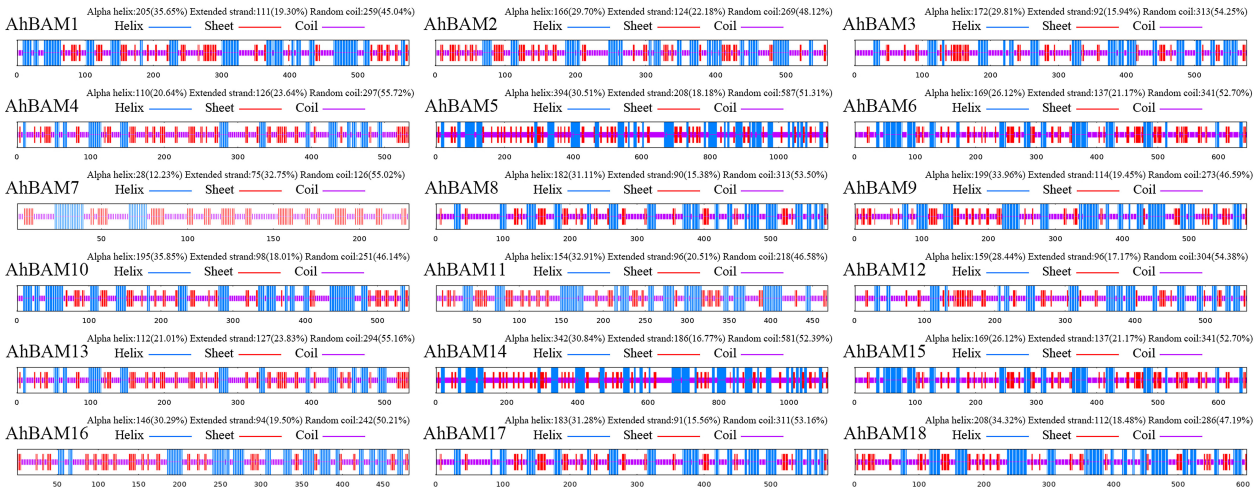

FIGURE 10
Secondary structure prediction of AhBAM proteins.

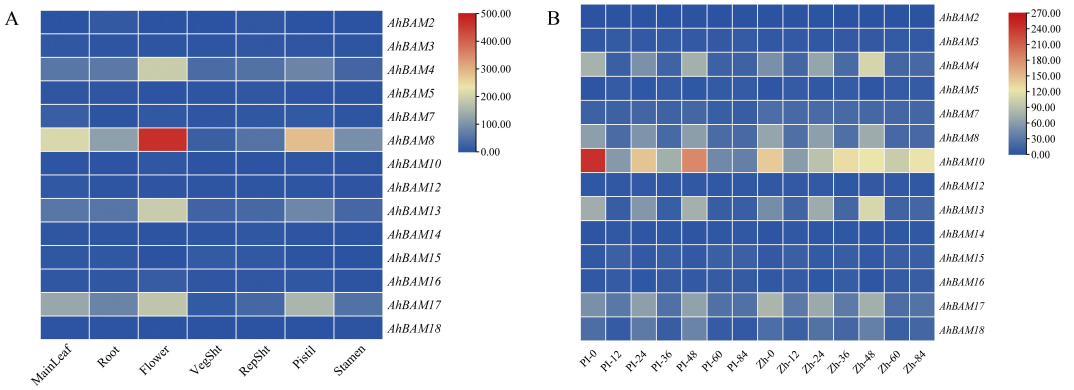

FIGURE 11
Expression profiles analysis of the peanut *BAM* gene family. **(A)** Heatmap of *AhBAM* gene expression in different peanut tissues. **(B)** Expression patterns of *AhBAM* genes under web blotch infection, as determined by RNA-seq.

between peanut and other species. This not only confirmed their close evolutionary relationship but also provides new insights into the adaptive evolution of leguminous plants. These conserved duplicated gene pairs in peanut and soybean may have driven functional diversification of *BAM* genes through processes such as subfunctionalization, thereby conferring species-specific metabolic regulatory features.

Functional diversity of the *BAM* gene family in peanuts

A comprehensive analysis of the *AhBAM* gene family in peanuts reveals its potential functions in plant growth, stress response, and metabolic regulation. Promoter analysis demonstrated that *AhBAM* genes are enriched with light-responsive (e.g., Box4), hormones-responsive, and abiotic stress-related cis-elements. This characteristic aligns with the regulatory patterns of *BAM* genes in tomato and sweet potato (Li et al., 2024; Huang et al., 2021), indicating conserved functions of this family in the crosstalk between light signaling, hormone responses, and stress adaptation. AhBAM3, positioned as a hub node in the protein-protein interaction network, likely integrates stress signals (e.g., SA and JA) with starch metabolic pathways to coordinate carbon allocation and energy homeostasis. This central regulatory feature closely resembles the function of Arabidopsis AtBAM3, suggesting that AhBAM3 may play a similarly critical role in peanut stress responses. These findings suggest that AhBAM3 subfamily members may have evolved analogous stress adaptation strategies in both leguminous and solanaceous plants during evolution, making them valuable molecular targets for improving stress resistance in crops. Exploring the regulatory mechanism of AhBAM3 could provide new insights into metabolic reprogramming and stress signaling in peanuts, offering potential applications in breeding stress-resilient varieties.

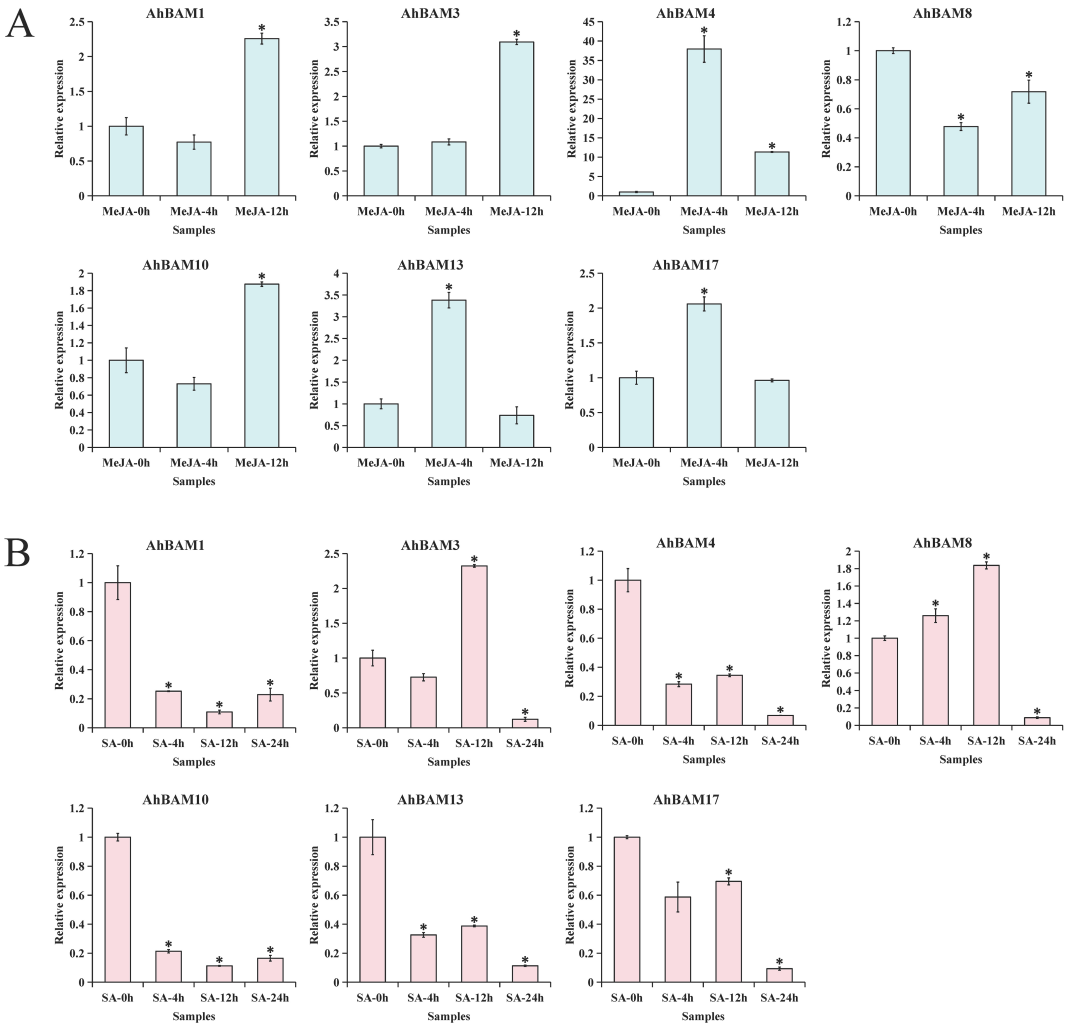

FIGURE 12
qRT-PCR validation of 7 *AhBAM* candidate genes induced by MeJA **(A)** and SA **(B)**. * indicates statistical significance at $p < 0.05$.

The three-dimensional structure directly influences the function of BAM genes

Comprehensive analysis of PTMs and structural features in the AhBAM protein family reveals critical insights into their regulatory mechanisms and functional diversity in peanut. The abundant tyrosine phosphorylation in AhBAM1 and AhBAM5 may regulate their enzyme activity and protein-protein interactions. Additionally, this mechanism is particularly relevant to stress tolerance in peanuts. Moreover, the identification of acetylation and methylation in multiple AhBAMs further underscores the regulatory complexity of this family, as these PTMs can alter protein stability and subcellular localization, ultimately influencing starch degradation efficiency during seed development or stress adaptation (Yang et al., 2025). Structurally, the conserved sequence motifs and predicted three-dimensional folds highlight both functional conservation and diversity within the AhBAM family (Foerderer et al., 2022). The high similarity among AhBAM1, AhBAM3, and AhBAM9 suggests similarity function in maintaining basal starch metabolism, whereas the distinct features of AhBAM2) may reflect specialized functions, such as tissue-specific expression. The PTM-driven regulation of AhBAMs could be exploited to enhance stress resilience or starch accumulation in peanut seeds. Future studies should validate these modifications in planta, particularly under field-relevant conditions, to harness their potential for peanut breeding programs.

AhBAM is likely to possess multiple biological functions, particularly in responding to environmental stimuli

Transcriptome data analysis revealed distinct spatial regulation of peanut *AhBAM*, with *AhBAM8* exhibited high expression in photosynthetic (main stem leaves) and reproductive tissues (flowers and pistils), while *AhBAM4/13/17* showed flower-specific expression patterns. This tissue-specific partitioning strongly implicates certain *AhBAM* isoforms in floral development and reproductive processes.

Peanut web blotch disease can occur throughout the entire growth period, often causing extensive leaf drop and severely affecting peanut yield and quality (Zhang et al., 2023). Most *AhBAM* genes showed no significant response to web blotch disease, except for *AhBAM10*, which exhibited moderate upregulation indicates that AhBAM-mediated starch metabolism may not be a primary defense mechanism against this pathogen. The distinct hormonal response patterns of AhBAM genes further emphasize their functional diversification. The early upregulation of *AhBAM4*, *AhBAM13*, and *AhBAM17* under MeJA suggests their involvement in jasmonate-mediated stress responses, possibly linked to wound healing. In contrast, the late induction of *AhBAM1*, *AhBAM3*, and *AhBAM10* implies roles in prolonged stress adaptation, such as drought. Importantly, *AhBAM3* emerged as a unique candidate due to its SA-specific upregulation, hinting at its potential role in balancing JA-SA crosstalk. The downregulation of multiple *AhBAM* genes under SA at early stages may reflect a trade-off between growth and defense. The expression dynamics of *AhBAM* genes underscore their tissue-specific and hormone-regulated roles in peanut development and stress adaptation.

Conclusions

This study comprehensively analyzed the *BAM* gene family in peanuts, identifying 18 members unevenly distributed across 10 chromosomes. Subcellular localization showed most members in the nucleus and chloroplasts. Phylogenetic analysis grouped them into four subgroups with conserved gene structures and motifs. Promoter analysis revealed enrichment of cis-acting elements related to light, hormones, and stress responses. Intraspecific collinearity identified eight gene pairs under purifying selection, while interspecific collinearity with soybean highlighted 18 gene pairs, indicating close evolutionary relationships. Network analysis pinpointed AhBAM3 as a central hub in signal integration. Gene Ontology analysis confirmed functional diversity, mainly in starch and glucose metabolism. Epigenetic and structural predictions showed both conservation and diversity within the family. Transcriptome data highlighted roles in flowering and MeJA/SA responses but not in web blotch disease resistance. These findings provide a solid foundation for further elucidating the molecular mechanisms and functional roles of the peanut *BAM* gene family.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.peanutbase.org/, arahy.3VMA9Y, arahy.NQ88PQ, arahy.U96YPL, arahy.I36EUT, arahy.UANN5Y, arahy.K0N155, arahy.68PBWJ, arahy.4Y2508, arahy.C3I2PC, arahy.34GL3D, arahy.0V89VS, arahy.ABG1ZW, arahy.60DV95, arahy.M409IX, arahy.Y0TSMC, arahy.JJTS0M, arahy.TP5BL7, arahy.Y6Z03A.

Author contributions

CS: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. PL: Writing – original draft, Formal analysis, Data curation. LL: Data curation, Formal analysis, Writing – original draft. ZQ: Writing – original draft, Data curation, Formal analysis. TL: Writing – original draft, Formal analysis, Data curation. JS: Data curation, Writing – original draft, Formal analysis. HY: Writing – original draft, Visualization. JW: Visualization, Writing – original draft. JG: Visualization, Writing – original draft. ML: Writing – original draft, Visualization. XW: Supervision, Funding acquisition, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This research was funded by the Henan Provincial Natural Science Foundation (Project No.: 252300420221), Henan Province Science and Technology Research Project (Project No.: 252102110270), Doctoral fund project of Zhengzhou University of Light Industry, China (Project No.: 2024BSJJ011).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2025.1599610/full#supplementary-material

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